METHODS FOR FLUORESCENCE DETECTION THAT MINIMIZES UNDESIRABLE BACKGROUND FLUORESCENCE

FIELD OF THE INVENTION

[0001] This invention relates generally to fluorescence analytical techniques. More specifically, the invention relates to a method and apparatus for detecting a fluorescent sample that minimizes undesirable background.

BACKGROUND OF THE INVENTION

[0002] Fluorescence detection is widely used in biochemical and medical research applications due to its high sensitivity. For example, fluorescence detection is used in automated DNA sequencing, capillary electrophoresis and a variety of immunoassays. In response to excitation, fluorescent biomolecules and dyes emit light at characteristic wavelengths, which differ from the excitation wavelength. By detecting these characteristic wavelengths, the composition of a sample can be determined.

[0003] In many biological applications, the amount of sample to be detected is usually quite small. Over the years, methods and apparatus have been able to manipulate and separate on smaller and smaller scales, going from the μM range to nM and pM ranges. As the sample size decreases, the background fluorescence becomes more significant in relation to the fluorescence of the sample.

[0004] The dominant background noise source in fluorescence detectors is often shot noise. Shot noise comes from the sample and

background fluorescence. The background fluorescence comes from fluorescence or Raman scattering from the sample as well as from the substrate that the sample is contained in. High background fluorescence also reduces the dynamic range of the detector by causing saturation of the detector. Therefore, reducing the background noise is one strategy for improving the performance of fluorescence detectors.

SUMMARY OF THE INVENTION

[0005] The present invention provides a method for the excitation of a fluorescent sample and the measurement of the fluorescent emission that significantly reduces the amount of background fluorescence. The method includes the steps of exciting a sample in a substrate with a beam of light that enters the substrate at an angle less than or equal to about 45°, and more preferably, less than or equal to about 20° and collecting the fluorescent emission from the sample with a lens system which focuses the emitted light onto a charge coupled device (CCD) for detection.

[0006] The beam of light is generated from a laser and is directed to the sample-containing substrate by a scanning mirror and a prism. The light enters the substrate at an angle less than or equal to about 45°, and more preferably, less than or equal to about 20° with respect to the axis of the channel plates and continues through the channel plate into the sample. In another embodiment, a lens system collects and collimates the fluorescence emitted by the excited sample. The collected light then passes through a wide bandpass

filter to exclude scattered laser light. The collected light then passes through a transmission grating which disperses the light in the spectral axis, which is oriented perpendicular to the axis of the substrate. The image is then focused onto a scientific grade CCD for detection.

[0007] The method of the present invention can be used with a scanning system or a non-scanning system. Non-limiting examples of application of the present invention are scanning systems using channel plates and capillary systems such as capillary electrophoresis.

[0008] Further areas of applicability of the present invention will become apparent from the detailed description provided hereinafter. It should be understood that the detailed description and specific examples, while indicating the preferred embodiment of the invention, are intended for purposes of illustration only and are not intended to limit the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and subjoined claims and by referencing the following drawings in which:

[0010] Figure 1 is a schematic block diagram illustrating a fluorescence detection system; and

[0011] Figure 2 is a photograph showing the CCD image generated following exposure to an excitation beam of light.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0012] The present invention provides a method for the excitation of a fluorescent sample and the measurement of the fluorescent emission. The method includes the steps of exciting a sample in a substrate with a beam of light that enters the substrate at an angle less than or equal to about 45°C , and more preferably, less than or equal to about 20°, and then collecting the fluorescent emission from the sample with a lens system which focuses the emitted light onto a CCD for detection. Although the following description of the present invention uses a scanning system using channel plates by way of illustration, the method described herein may also be used with non-scanning systems as well as capillary systems.

[0013] In one embodiment of the invention, the excitation beam is directed to the channel by a scanning mirror and prism. The excitation beam can be generated by a UV, visible or infrared light source, preferably by a laser. The angle of the mirror can be adjusted to control the angle that the excitation beam enters the channel. Preferably, the excitation beam enters the channel at an angle less than or equal to about 45°, and more preferably, less than or equal to about 20°. The optimal angle for the incident beam will depend on the index of refraction of the material of the channel plate. Generally, the more shallow the angle, the greater the amount of sample fluorescence collected along with a concomitant reduction in the background fluorescence.

[0014] In another embodiment of the present invention, collection optics collects and collimates the fluorescence from the excited sample into

parallel rays. Preferably the lens is situated perpendicular to the channel axis. The collecting lens may be a simple camera lens. The collected light then passes through a long pass or wide bandpass filter, which removes scattered light at the laser wavelength. The remaining filtered light, which consists essentially of fluorescence from the sample and background from the channel, then passes through a transmission defraction grating. The transmission grating separates the light into rays of differing wavelength that diverges along the direction of the spectral axis, perpendicular to the channel axis. Finally a focusing lens directs the light onto the CCD.

[0015] In a further embodiment, the image from the CCD is collected to bin or read out as a data file. Preferably, only the section of the image on the CCD associated with the fluorescence of the sample is collected by selection of the appropriate pixels to bin and read out. In the method of the present invention, as the excitation beam moves through the plate at an angle, the excitation beam creates a fluorescent trail. When this trail is imaged by the collection optics, the fluorescence from different parts of the channel will fall on different sections of the spatial axis of the CCD. As illustrated in Figure 2, the fluorescence associated with the sample is separated from the background fluorescence

[0016] An apparatus for performing the above-described methods of the present invention is shown in Figure 1. Figure 1 illustrates an apparatus 10 for use with a scanning system using a channel plate 12. The channel plate 12 defines a channel 14 which receives a medium that contains samples 16. A

current is applied to the medium that contains the samples 16 by means of a pair of electrodes 18. Upon passing a current through the medium, the samples 16 are separated as is known in the electrofluorescence art. The channel plate 12 may be formed from glass, fused silica, plastic or other transparent type material. The channel plate 12 is supported by a support plate 13 formed from glass, fused silica, plastic or other transparent material. In addition, the channel 14 may be defined by other suitable structures such as capillary tubes, arrays of capillary tubes and slab gel with field defined lanes.

[0017] A laser 20 generates an excitation beam 22 that is essentially parallel to the channel plate 12 and directed toward a reflective mirror 24. The mirror 24 is adjusted to reflect the excitation beam 22 at the desired angle into the channel plate 12. Here again, the excitation beam 22 enters the channel plate 12 at an angle less than or equal to about forty-five degrees (45°), and preferably less than or equal to about twenty degrees (20°). The excitation beam 22 is directed through a prism 26 to facilitate entry of the excitation beam 22 into the support plate 13. The support plate 13 is optically coupled to the channel plate 12 using water, direct contact or any transparent material with an index similar to the channel plate. The focused excitation beam 22 enters the channel plate 12 and passes through the channel plate 12 before reaching the channel 14 containing the sample 16. The focused excitation beam 22 continues through the top layer of the channel plate 12. As defined by the Fresnel Equations, some light is reflected at boundaries where the index of refraction changes. This creates the reflected beams 28. Both the focused excitation beam 22 and the

reflected beam 28 can generate undesirable fluorescent emissions from the samples 16.

[0018] A portion of the sample fluorescence emissions 30 enters collection optics 32 where the emitted light is collected, collimated and dispersed before being focused onto CCD 34 using known optics and CCD technology. In this regard, the collection optics 32 includes a first collimating lens, which collects and collimates the fluorescence from the excited sample 16 into parallel rays. The collected light is then passed through a long pass or laser rejection filter, which removes scattered or stray light at the laser wavelength(s). The remaining filtered light, which consists of the fluorescence emissions from the sample 16 and fluorescent background from the channel plate 12 is then passed through a transmission defraction grating, a grism, a prism or reflected off a reflective grating. The transmission defraction grating separates the lights into rays of differing wavelengths that diverges along the direction of the spectral axis which is perpendicular to the axis of the channel 14. Finally, a second focusing lens focuses the light onto the CCD 34. The collection optics 32 may be similar to the optics system disclosed in , United States Serial No. 09/564,790 filed May 5, 2000 or Simpson et al., "A Transmission Imaging Spectrograph and Micro fabricated Channel System for DNA Analysis", Electrophoresis 2000, 21, 135-149, both of which are hereby incorporated by reference. However, other suitable optics systems may be used. Further, it will be appreciated other types of detectors can also be used to receive light from the sample 16. These include CMOS detectors, photodiodes, photodiodes arrays, photomultiplier tubes,

photomultiplier tube arrays or other suitable detectors. In addition, the preferred orientations of the collection optics 32 is substantially perpendicular to the excitation beam 22 entering the sample 16 as this allows a larger amount of light from the sample to be collected while still rejecting background.

[0019] The image from the CCD 34 is collected to be read out as a data file as is shown and illustrated in Figure 2. In this regard, Figure 2 illustrates both the glass fluorescence 36, which is the background fluorescence from the channel plate 12, as well as the collected light 38 from within the channel 14 which consists of the fluorescence from the sample 16. This collected light 38 is preferably from the only section of the image on the CCD 34 associated with the fluorescence of sample 16, which is collected by selection of the appropriate detector elements or pixels (which receive little background fluorescence) from which meaningful data is to be read. The CCD 34 may also use "binning" in which the photogenerated charge in adjacent detector elements are read out as a combined charge pocket during a single read. The use of binning may reduce overall noise when compared to other processing techniques, but may be accompanied by loss of spacial resolution.

[0020] By allowing the excitation beam 22 to enter the channel plate 12 at an angle of less than about forty-five (45°), and preferably less than or equal to about twenty degrees (20°), much of the reflected light and background fluorescence that is produced by the excitation beam 22 entering the channel plate 12 is directed away from the collection optics as is shown in Figure 2. Accordingly, because less background noise enters the collection optics 32; the

sensitivity of the apparatus 10 is increased. In addition, because the excitation beam 22 enters the plate at a relatively small angle with respect to the collection plate 12, a greater amount of fluorescent light is created by the samples 16 causing an increase in the fluorescence of the sample and therefore improved sensitivity.

[0021] The method for selecting which pixels or detector elements are used to generate meaningful data from which spectral information is to be determined, and which pixels or detector elements should be ignored as receiving excessive background fluorescence, involves two considerations. The signal-to-noise ratio of the CCD 34 should be maximized while the dynamic range of the CCD 34 is not excessively limited. One method for selecting which detector elements should be used to generate meaningful data for analysis is as follows.

[0022] First, the output from a first group of detector elements near the center of the image on the CCD 34 is recorded and the signal-to-noise ratio determined. Once the signal-to-noise ratio has been determined from this first set of detector elements, the signal-to-noise ratio is compared to the signal-to-noise ratio from a second group of detector elements. This second group of detector elements includes those detector elements in the first group as well as detector elements that are adjacent to the detector elements in the first group. If the signal-to-noise ratio increases, this indicates that better data can be obtained if the second group of detector elements is used to generate spectral information as compared to using the first group of detector elements. The output of the

second group of detector elements is therefore initially selected to be used to generate meaningful data from which spectral information is to be determined.

[0023] This process is continued with progressively larger sets of detector elements until the signal-to-noise ratio begins to decline. When the signal-to-noise ratio begins to decline, the inclusion of additional detector elements does not improve collection of meaningful data and therefore the output from the remaining detector elements is not considered. However, during this process, care must be taken that the background noise does not consume so much of the capacity of the CCD 34 so as to diminish the dynamic range of the CCD 34. If too much of the dynamic range is consumed a lower number of bins should be used.

[0024] The foregoing description discloses and describes merely exemplary embodiments of the present invention. For example, the excitation beam 22 could enter the channel plate 12 at an angle greater than 45° if the collection optics 32 is located off-axis (i.e., off-set from the direction perpendicular to the direction of the excitation beam 22 entering the sample). Further, the inside top surface of the channel plate 12 may be coated with a low index material or fabricated from a low index material (e.g., Teflon AF). In such a case, the excitation beam 22 could be orientated at an angle (i.e., about 22°) that would be totally internally reflected so as to further separate the detection region from the background region. Further, multiple excitation beams 22 may be used as well as multiple detection elements. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings that

various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention.